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TO: Chair, Pesticide Board Subcommittee

FROM: Elizabeth Anne Bourque, Ph.D. and Joel Swartz, Ph.D.

RE: Review of the Public Health Concerns of the Herbicide - Amitrole

DATE: December 28, 1984

At the October 31, 1984 Pesticide Board Subcommittee meeting, an outline of topics to be included in the analysis of amitrole was discussed.

The Subcommittee's concern about this product developed from the draft Generic Environmental Impact Report (GEIR) on the Control of Vegetation on Utility and Railroad Rights-of-Way in Massachusetts released in December 1983. This report examined the toxicity and environmental impact of fourteen herbicides. Amitrole was singled out as a high toxicity herbicide and carcinogen. The GEIR therefore recommended that amitrole be "considered for removal from the list of acceptable herbicides to be used on rights-of-way" (Harrison Biotech, 1983).

Recent steps taken to address the concern over amitrole have been:

1. June 4, 1984, Massachusetts Pesticide Board Subcommittee decided to have the staffs from the Pesticide Bureau of the Department of Food and Agriculture and the Center for Health Promotion and Environmental Disease Prevention conduct a comprehensive review of the use of amitrole in Massachusetts.

2. August 31, 1984, Notification of the Intention to Conduct a Comprehensive Review of the Use of Amitrole in Massachusetts, from Jeffrey L. Carlson, Pesticide Bureau Chief, to Registrants of Amitrole-Containing Pesticide Products which are currently registered for use in Massachusetts.

Therefore, the Department of Public Health, Center for Health Promotion and Environmental Disease Prevention has collected and reviewed the pertinent health information available for amitrole. Alternatives to amitrole were not considered in this review. This review appears as Sections II - XI. A risk assessment has also been developed and appears as Section IX.

REVIEW OF THE PUBLIC HEALTH CONCERNS O. AMITROLE

	page
I. SUMMARY	1
II. CARCINOGENCITY	4
III. MECHANISM OF ACTION	6
IV. METABOLISM	8
V. ABSORPTION	9
VI. ACTIVITY IN SHORT-TERM TESTS	10
VII. ANIMAL STUDIES	11
VIII. HUMAN STUDIES	25
IX. RISK ASSESSMENT	27
X. PERSISTENCE IN SOIL AND WATER	39
XI. BIBLIOGRAPHY	40

I. SUMMARY

The public health concerns regarding amitrole focus on the following aspects:

A. Amitrole is a carcinogen.

Data from three types of studies: short-term tests, animal studies and human studies are usually considered in determining whether or not a substance is a carcinogen.

1. Short Term Tests

Amitrole was mutagenic in fungi and produced cell transformation in Syrian hamster embryo cells. Although it was negative in the Salmonella assay and Escherichia coli pol A system, amitrole gave positive results in DNA-Cell-Binding assay employing Escherichia coli and a host-mediated testing response in Salmonella typhimuruium. Some carcinogens may not be detectable as mutagens in the present in vitro systems. This group includes thyroid active compounds (Bartsch, H. Tomatis, L. and Malaveille, C. 1982). It is not surprising then that as a chemical which exerts some of its effect on the thyroid, amitrole has not always given positive results in short-term tests.

2. Animal Studies

There is a remarkable consensus among expert agencies and committees Environmental Protection Agency (EPA), National Toxicology Program (NTP), International Agency for Research on Cancer (IARC) and Carcinogen Assessment Group (CAG) that amitrole is a definite carcinogen which induces thyroid and liver tumors in two species of animals. These groups have recommended that in the absence of adequate data in humans, it is reasonable for practical purposes to regard amitrole for which a causal association in two species has been seen, as if it presented a carcinogenic risk for humans. One animal study used amitrole as a positive control to determine if 120 other chemicals were carcinogens (Innes, J. et al., 1969). Although it has been claimed that the effect of amitrole on the thyroid is reversible,(Hazelton Laboratories, 1959) there is no evidence in this study to support that claim.

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3. Human Studies

There have been three human studies in occupationally exposed groups. Swedish railroad workers exposed to amitrole and other herbicides were the subject of two mortality studies. The first study showed a slight but statistically significant excess of cancer when all sites were considered together. Since there was exposure to several different herbicides, it is not possible to evaluate the observed excess tumor incidence as clearly related to amitrole (Axelson, O. and Sundell, L. 1974). The second study extended the follow-up time of the first. Once again, the exposure was to several herbicides and there was an excess of all sites of tumors (Axelson, O. et al., 1980). These small cohort studies do not rule out that amitrole may be carcinogenic to man. The third human study included medical monitoring and assessment of exposure of 5 applicators carried out over 10 work days (Dynamac Corporation, 1984). While the study showed an increase in thyroid stimulating hormone (TSH) level, which may lead to neoplasia, the number of subjects and the length of exposure are inadequate to demonstrate statistical significance. However, the relative risk for humans exposed during a typical application has been calculated from the animal data and found to be very high.

B. Risk Assessment for Amitrole

Amitrole acts in all respects like a classical, complete, direct acting, genotoxic carcinogen. Therefore, its carcinogenic action at high doses indicates that it is also carcinogenic at much lower doses. There is no evidence of a threshold for the carcinogenic action of amitrole. Even if the existence of a threshold could be shown on theoretical grounds, there is no available technique for determination of a threshold.

There is a plausible case that amitrole causes thyroid and pituitary tumors through an indirect method by interfering with the synthesis of thyroid hormones, and that there is therefore a threshold for this carcinogenic action. However, amitrole also induces liver tumors, sarcomas, and tumors of other sites. It tests positively in short term assays for various forms of mutagenicity and transformation in cell culture. Together these indicate that amitrole should be treated as a classical carcinogen with no threshold of activity.

A risk assessment was made for amitrole using the one hit, or linear no threshold model. Of all the bioassays on amitrole only the one by Vesselinovitch met the conditions to be used for risk assessment. In the remaining bioassays either the dose was so high that risk assessment was confounded by competing causes of death, or too few animals were at risk. Extrapolation between species was made using dose of amitrole per surface area per day as the appropriate equivalent units. The projected risk for applicators for lifetime exposure ranged from 0.31 for regular clothing and 100% dermal absorption to 0.028 for protective clothing and 30% dermal absorption. These risks are high, but the exposure to applicators is very high. Risks are accordingly many orders of magnitude lower for persons with incidental contact with amitrole.

C. Amitrole is absorbed and distributed throughout the body.

Animal studies show that amitrole is rapidly absorbed dermally, as well as, through inhalation and via the gastrointestinal tract. It is transported throughout the body and metabolized to a small extent in the liver. The main excretion product at 24 hours is amitrole.

D. Amitrole is persistent in soil and transported by water.

Amitrole persists in soil for about a month. It persists in water for 200 days and is transported by water.

Actions for consideration for Subcommittee

1. Allow products to retain General Registration, or

2. Restrict use to certified applicators, or

3. Restrict use to certified applicators with the imposition of additional Conditions of Registration, or

4. If 1, 2 or 3 are effected by the Subcommittee, a recommendation should be considered for the development of additional regulations by the Department of Food and Agriculture, or

5. Revoke or cancel the Registration.

II. CARCINOGENICITY

The Environmental Protection Agency (EPA) notified registrants on June 16, 1971 that all formulations containing amitrole and "bearing use directions for food crops would be cancelled because this chemical was a carcinogen in experimental animals" (EPA, March 1984). The EPA initiated a Special Review of Amitrole in 1984. It determined that amitrole is oncogenic in the thyroid, pituitary and liver of laboratory animals. EPA further determined that:

> "...amitrole caused oncogenic effects in laboratory animals and that applicator exposure and oncogenic risk were high." (49 Fed. Reg. 20546, May 15, 1984)

The Massachusetts Right-to-Know list included amitrole as a carcinogen. Several other national and international agencies have examined the carcinogenicity of amitrole.

The National Toxicology Program (NTP) listed amitrole as a substance".. that maybe reasonably anticipated to be a carcinogen." piii (National Toxicology, 1983). This agency stated that:

> "There is sufficient evidence of the carcinogenicity of amitrole in experimental animals; the evidence of carcinogenicity in humans is considered inadequate. Amitrole was carcinogenic in several strains of mice and rats, producing thyroid and liver tumors following administration in drinking water, diet, or by stomach tube, or subcutaneous injection." p20 (National Toxicology Program, 1983).

The Carcinogen Assessment Group (CAG) prepared a list of chemical substances for which substantial or strong evidence exists that exposure to these chemicals under certain conditions "causes cancer in humans, or can cause cancer in animal species which in turn, make them potentially carcinogenic in humans" p1 (Carcinogen Assessment Group's List of Carcinogens, 1980). Amitrole was listed as a chemical having substantial evidence of carcinogenicity. The group further clarified the term substantial:

> "... has been used to cover carcinogenic evidence ranging from single, clear-cut responses in animal studies to stronger evidence in multiple species and results from human studies." p1 (Carcinogen Assessment Group's List of Carcinogens, 1980).

The American Conference of Industrial Hygienists (ACIH) listed amitrole as a substance suspect of carcinogenic potential in man. This classification is based upon

> "... either (1) limited epidemiologic evidence, exclusive of clinical reports of single cases, or (2) demonstration of carcinogenesis in one or more animal species by appropriate methods" p42 (American Conference of Industrial Hygienists, 1983-1984).

The International Agency for Research on Cancer (IARC) Presently, no objective criteria exist to interpret data from studies in experimental animals or short-term tests directly in terms of human risk. In the absence of sufficient evidence from human studies, evaluation of carcinogenic risk to humans was based on consideration of experimental evidence. IARC considered a combination of sufficient evidence in animals and inadequate data in humans as the criteria for classifying substances as probably carcinogenic to humans (IARC, 1982).

The Commonwealth of Massachusetts, Generic Environmental Impact Report was prepared to develop guidelines for the use of herbicides on rights-of-way. As a result of the review of data, the report stated that amitrole was in the high-toxicity category and of the 14 herbicides considered, only amitrole, "as a carcinogen, clearly belongs in this category" p81 (Harrison Biotech, 1983). The report clearly recommended:

> ". . . aminotriazole, should be considered for removal from the list of acceptable herbicides to be used on rights-of-way in Massachusetts" p81 (Harrison Biotech, 1983)

There is no doubt that amitrole causes cancer in animals. When precise answers are not available, agencies should consider making decisions on the best available evidence. Several agencies listed above have concluded that amitrole is reasonably anticipated to be a carcinogen. The statements made concerning amitrole in this context are:

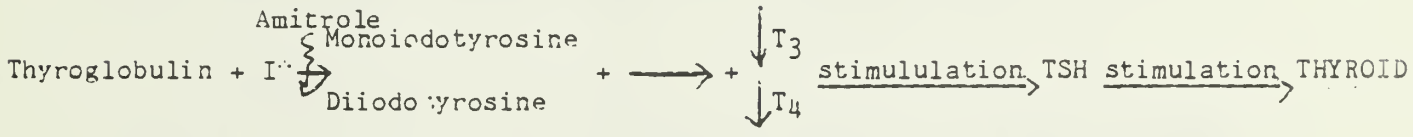
1. Applicator exposure and oncogenic risks were high (EPA),
2. Reasonably anticipated to be carcinogenic in humans (NTP)
3. Potentially carcinogenic in humans (CAG)
4. Suspect of carcinogenic potential in man (ACIH)
5. Probably carcinogenic in humans (IARC)

The evidence points to amitrole as a human carcinogen.

III. MECHANISM OF ACTION

A. Inhibition of Thyroid Peroxidase

Amitrole acts on the thyroid gland by inhibiting thyroid peroxidase, an enzyme essential in the synthesis of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). This mechanism appears to be similar in rodents and man.



The synthesis of triiodothyronine (T_3) and thyroxine (T_4) involves the iodination of tyrosine by thyroid peroxidase to form monoidotyrosine and diiodotyrosine which in turn couple to form T_3 and T_4. The decreased thyroid hormone level produced by amitrole increases pituitary secretion of thyroid stimulating hormone (TSH) which stimulates the thyroid gland to compensate for the decreased hormone level.

The thyroid not only synthesizes but stores and secretes as necessary the thyroid hormones. The secretion of these hormones is regulated by thyroid stimulating hormone (TSH), which initially is regulated by the amount of T_3 and T_4 circulating in the blood. In other words, there is a circulatory feedback mechanism of control of the thyroid hormones. As with other hormones, T_3 and T_4 are transported in the blood to the target organs. The primary effect is believed to be stimulation of protein synthesis, which results in production and utilization of energy. These hormones also play a vital role in the growth, differentiation, and development of the individual. Clearly, the inhibition of thyroid peroxidase by amitrole not only affects the thyroid gland by interfering with T_3 and T_4 synthesis and secretion but also the general metabolism of an individual.

The effects of thyroid peroxidase inhibition are threefold. First, the resulting decrease in T_3 and T_4 will produce overstimulation of the thyroid gland which will induce enlargement of the thyroid gland. This condition can lead to neoplasia. (Environ Corp, 1984).

Secondly, the decreased level of thyroid hormones will alter the cellular reactions in practically every organ and tissue in the body. It will affect the basal metabolic rate, growth and O_2 consumption of the whole organism. On the molecular level, decreased thyroid hormone levels will interfere with protein synthesis resulting in altered rates in more than 100 enzymic systems (White, A., Handler, P. and Smith, E. 1973).

Thirdly, hypothyroidism is associated with significant changes in renal function in humans, as well as in experimental animals following drug induced hypothyroidism. A study of kidney structure of rats fed amitrole 500mg/g feed for four weeks shows decrease in kidney weight due mainly to a reduction of cortical tissue. There is a decrease in the peritubular diameters of both the proximal tubules and the thick ascending limb of the distal convoluted tubules. A decreased cell height in the thick ascending limb of the distal convoluted tubules is also seen. These amitrole induced changes in the kidney could be prevented by simultaneous treatment with T_4 (Davis, R.G. et al., 1983).

B. Inhibition of Other Enzymes

Mice injected twice-daily with amitrole (1mg/g b.w.) and fed pellets containing 1% amitrole have significantly lower (14% of normal) catalase activity (Jones, G.L. and Neill, A.R., 1982). Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase elimination of hydrogen peroxide is important as this compound is extremely reactive and capable of irreversible damage to various biomolecules (Lehninger, A., 1975)

Other studies have shown that amitrole inhibits heme biosynthesis in mouse and rat liver. As a consequence of this inhibition, amitrole also inhibits adipose differentiation of mouse 3T3 cells to adipocytes in vitro. This mechanism is confirmed by the simultaneous addition of hemin and amitrole which prevents the inhibition of adipose differentiation. (Chen, J.J. and London, I.M., 1981).

It can be seen that inhibition of thyroid peroxidase by amitrole would have effects throughout the body.

IV. METABOLISM

The main excretion product of a single oral dose of ^{14}C-amitrole to rats is amitrole. Small amounts of total 24 hour urine radioactivity are two metabolites, metabolite-1 (25%) and metabolite-2 (1%). The liver is the site of metabolite-1 formation. Extracts of all tissue except liver and kidney contain mainly amitrole with a trace of metabolite-1. The removal of metabolite-1 from liver is much slower than removal of amitrole and is similar to the rate of decrease of catalase inhibition. The authors state that metabolite-1 might be the toxic agent responsible for liver catalase inhibition. (Fang, S.C., Geroge, M. and Yu. T.C., 1964).

Human metabolism of amitrole appears to be similar. A urine sample taken some hours after a 39 year old woman ingested 20 mg/kg of amitrole contained unchanged amitrole. No metabolites were found. (Menzie,C.M., 1978 cited in Toxicology Data Bank).

V. ABSORPTION

Animal studies show that amitrole can be absorbed dermally, through inhalation or via the gastrointestinal tract.

1. Absorption of Aerosilized Compounds from the Rat Lung

An investigation of aerosolized amitrole inhaled through a trachael cannula demonstrates that 50% of the compound is absorbed by the lung in 1.3 minutes. The experimental design included 12 nonvolatile drugs of diverse physicochemical properties. The authors considered procainamide (50% absorption in 2.3 minutes) to be rapidly absorbed and p-aminohippuric acid (PAH)(50% absorbion in 21.7 minutes) as a slowly absorbed compound. The mass median aerodynamic diameter (MMAD) of amitrole in this study is 3.03 $\pm$ 0.09 µm. Numerous studies on inhaled aerosols in humans have indicated good alveolar penetration is achieved if droplets have a diameter of about 3 µm or less. Kliment has compared rat, guinea pig, rabbit and man; he showed that the degree of similarity in these species is high for deposition in the pulmonary region of the lung as well as for total lung deposition (Brown, R.A. and Schanker, L.S., 1983). This study demonstrates that amitrole is rapidly absorbed through the rat lung. One can conclude that due to the size of the droplet and the similarity of deposition within the lung similar absorption would occur in man.

2. Dermal Absorption in Rabbit

Rapid dermal absorption of ^{14}C-amitrole occurs in the rabbit. Amitrole was detected in the blood one minute after cutaneous application (1.1mg/kg · b.w.). After 15 minutes the order of penetration into the blood of the six pesticides studied was amitrole>carbaryl = parathion>malathion>DDT>dieldrin. A tissue distribution study showed that 24 hours after topical application amitrole was present in the 15 tissues examined. The liver contained 10% and the urine 26% of the amitrole assayed in the tissues. The thyroid was not examined. Moreover, 30% of the applied dose was absorbed. (Shah, P.V. and Guthrie, F.E., 1977) It is evident that amitrole is rapidly absorbed through the skin and is distributed throughout the body.

3. Absorption in the Gastrointestinal Tract of Rat

A single oral dose (3.3mg/kg b.w.) of 3-amino 1,2,4-triazole-5-C^{14} was rapidly and almost completely absorbed from the gastrointestinal tract. It was distributed throughout the body and was present in 12 out of 14 organs analyzed one hour after administration. The highest levels of radioactivity were found in blood, kidney, heart and liver. The thyroid was not analyzed. Amitrole was not found in fat tissue or skin. The maximum concentration in most vital organs, was reached within one hour and remained at approximately the same level for the next few hours. The amitrole levels then started to decrease and with the exception of liver reached virtually zero in three days. During the first 24 hours, 82% of the radioactivity was detected in the urine in the form of free amitrole and two other metabolites. The liver is the site of metabolite-1-formation. No free amitrole is present in the liver after 24 hours. The removal of metabolite-1 from the liver is much slower than removal of amitrole. (Fang, S.C. George, M. and Yu, T.C.,1964).

It should be noted that amitrole is quickly distributed throughout the body. The deposition and metabolism of amitrole in the liver is important, as animal studies show that amitrole induces hepatomas.

VI. ACTIVITY IN SHORT-TERM TESTS

There is evidence to suggest that DNA damage is involved in the induction of many cancers. Assays for mutagenicity and various manifestations of chromosomal damage in organisms ranging from bacteriophages to mammals, all exploit the characteristic ability of carcinogens to cause DNA damage or chromosomal anomalies either directly or indirectly. "However, some carcinogens may act by mechanisms that do not involve DNA damage and thus would not cause such genetic effects" p9 (IARC, 1982). Moreover some carcinogens may not be detectable as mutagens.

> "Certain classes of compounds, however, may not be detectable as mutagens even with improvements in in vitro activation systems or increased sensitivity of genetic indicator organisms; these appear to include sex hormones, thyroid active compounds, tumor promoters, and physically acting agents." (emphasis added) p.51 (Bartsch, H., Tomatis, L. and Malaveille, C. 1982)

It is not surprising then that as a chemical which exerts some of its effects on the thyroid, amitrole has not always given positive results in short-term tests. However, amitrole is mutagenic and produces chromsomal aberrations in fungii (IARC, 1982). Further it causes morphological transformation in Syrian hamster embryo cells. (Inoue, K., Katoh, Y. and Takayoma, S., 1981). The significance of this morphological transformation is seen in the production of carcinomas in hamsters inoculated with cultures transformed by by other carcinogens in this same study (Pienta, R.J., Poiley, J.A., Lebherz, W.B., III, 1977).

A number of short-term tests for carcinogens employ as endpoints certain markers which exist in prokaryotes (e.g. bacteria). These cells do not possess, the range of enzyme systems known in intact mammals to metabolize unreactive carcinogens to reactive electrophiles. Therefore, it is often necessary to provide an exogenous source of such activity in the form of a tissue extract or an animal host. Amitrole gives positive results in the DNA-Cell-Binding assay. There is increased attachment of DNA to intact Escherichia coli Q13 in the presence of liver extract (Kubinski, H., Gutzke, G.E. and Kubinski, Z.O., 1981). Further, amitrole produces a mutagenic response in Salmonella typhimurium in the i.p. host-mediated assay. Equimolar amounts of sodium nitrite plus amitrole form a mutagenic product in the mouse stomach. (Braun, R., Schoneich, J. and Ziebarth, D., 1977)

However, amitrole is not mutagenic in the Salmonella assay, Escherichia coli pol A system or the Drosophila melanogaster (IARC, 1982). Although the evidence for amitrole activity in short-term tests is inadequate, some positive results have been obtained. These positive results should not be overlooked.

VII. ANIMAL STUDIES

The animal studies have been outlined in detail in Table I. Amitrole induced excess thyroid and liver tumors in both mice and rats following oral or subcutaneous administration.

A. Mice

Animals fed the maximum tolerated dose (1,000 to 2,192 ppm) developed excess hepatomas, significant at 0.01 level. Thyroid tumors were induced in 89% of the mice (Innes, J. et al.1969). At 500 ppm, 36% of the males and 18% of the females developed benign and malignant liver tumors. No thyroid tumors were reported as the thyroids were not examined (Vesselinovitch, S.D., 1983). In the third mouse study, animals were fed 1 ppm, 10 ppm or 100 ppm but the unusually high number of tumors seen in the control mice rendered it impossible to come to any conclusions about the effects of amitrole. (Steinhoff, S. et al., 1983)

B. Rats

Amitrole at 500 mg/rat/day, induced thyroid and liver tumors in 50% of the animals. (Napalkov, N.P., 1969 cited in IARC, 1974). Pellets and injections induced sarcomas at the treatment site of 50% of the animals (Napalkov, N.P. 1969 cited in IARC, 1974). Subcutaneous injections of amitrole induced thyroid and liver tumors in 71% of the animals (Napalkov, N.P., 1962 cited in IARC, 1974). At 10 or 50 ppm too few animals were at risk to draw any conclusions. At 100 ppm in the diet, thyroid adenomas were induced in 65% of the animals. (Jukes, T.H. and Shaffer, C.B., 1960). In another study rats were fed 1ppm and 10 ppm but the unusually high incidence of tumors in the control rats made it impossible to reach any conclusions. Whereas, at 100ppm there was a distinct increase in the number of pituitary and thyroid tumors, as well as a statistically significant, $p \leq 0.06$ decrease in lifespan. (Steinhoff, D. et al., 1983)

Two studies attempted to reverse the effect of amitrole. In the first study, T_4 was concurrently fed with amitrole and there appeared to be a decrease in thyroid tumors but there was no effect on the number of liver tumors induced (Napalkov, N.P., 1969). The second study claimed that the effect of amitrole, enlarged thyroids, was reversed when the animals were subsequently placed on a normal diet. However, the gross pathological findings showed that 7 weeks after the animals were placed on the control diet 79% had enlarged thyroids (Hazelton, Laboratories, 1959).

C. Hamsters

There was a statistically significant, $p \leq 0.0002$ shortening of hamster lifespan in animals fed 100 ppm amitrole. (Steinhoff, D. et al.,1983).

D. Summary

It is clear that only in the studies with poor experimental design, that is too few animals at risk, controls with unusual high incidence of tumors or statistically significant life-span shortening, were the induction of tumors by amitrole inconclusive. In all other studies an excess of thyroid and/or liver tumors were produced by amitrole.

Table I

VII. ANIMAL STUDIES: All the dosages have been converted to intake per surface area, mg/m^2 so that there is a common parameter for comparison among species.

1. Mice

a. Toxicity	LD_{50}	oral	14,700 mg/kg	
	LD_{50}	intraperitoneal	200 mg/kg	(Registry of Toxic Effects of Chemical Substances, 1980)

b. Carcinoma of Thyroid and Hepatomas In this study amitrole was one of the seven positive controls used to determine if 120 test compounds would induce tumors comparable to the mean incidence of the positive controls. Therefore, the initial premise in the experimental design was that amitrole is a carcinogen.

Strain	Number	Dosage (day)	Method	Age (days)	Dosage(day) mg/m^2
$(C57BL/6 \times C3H/Anf)_{F1}$	18m + 18f	1,000mg/kg	stomach tubing	7-28	
		2,192 ppm	diet,ad libitum	28-540	1.04×10^3
$(C7BL/6 \times AKR)_{F1}$	18m + 18f	1,000mg/kg	stomach-tubing	7-28	
		2,192 ppm	diet,ad libitum	28-540	1.04×10^3

Results:		Animals affected/Total animals	
Carcinoma of Thyroid:		64/72	
Hepatomas:	$(C57BL/6 \times C3H/Anf)_{F1}$	16/18m	18/18f
	$(C57BL/6 \times AKR)_{F1}$	16/18m	17/17f
Controls:		13/169m	1/169f

Conclusion: The mice were given the maximum tolerated dose of amitrole. The increased incidence of hepatomas was significant at 0.01 level. Thyroid tumors were found in 89% of the animals.

(Innes,J. et al.,1969)

c. Hepatocellular Adenomas and Carcinomas

Strain	Number	Dosage (day)	Method	Age	Dosage(day) mg/m^2
$B6C3F_1$	74m	500ppm	diet,ad libitum	pregnant females	2.6×10^2
	83f	500ppm	diet,ad libitum	12 day gestation to delivery	

Results: Hepatocellular Carcinomas

Animals affected/Tolal animals
4/74m
0/83f

Hepatocellular Carcinomas

2/74m
0/83f

Strain	Number	Dosage (day)	Method	Age	Dosage(day) mg/m^2
$B6C3F_1$	45m	500ppm	diet,ad libitum	mothers	2.6×10^2
	55f	500ppm	diet,ad libitum	from delivery to weaning	2.6×10^2

Results: Hepatocellular Carcinomas

Animals affected/Total animals
6/45m
0/55f

Hepatocellular Carcinomas

4/45m
0/55f

Strain	Number	Dosage (day)	Method	Age	Dosage (day) mg/m^2
$B6C3F_1$	55m	500ppm	diet, ad libitum	weaning	2.6×10^2
	49f	500ppm	diet, ad libitum	through 90 weeks	2.6×10^2

Animals affected/Total animals

Results: Hepatocellular Adenomas — 9/55m, 5/49f

Hepatocellular Carcinomas — 11/55m, 4/49f

		Age Animals Sacrificed
Controls: Hepatocellular Adenomas	0/100m, 0/99f	52 weeks
	1/98m, 0/96f	90 weeks
	4/100m, 1/100f	142 weeks
Controls: Hepatocellular Carcinomas	0/100m, 0/99f	52 weeks
	0/98m, 0/96f	90 weeks
	3/100m, 0/100f	142 weeks

Conclusion: Amitrole was ineffective in inducing liver neoplasia in prenatal or infant mice when the mice were fed amitrole over a very short period of time, 10 days and 28 days respectively. However, 36% of the males and 18% of the females fed amitrole for about 600 days developed benign and malignant tumors. As this was a study in hepatocarcinogenesis, only livers were examined. No other organs were analyzed, thryoids were not examined. Therefore, no thyroid tumors were found.

(Vesselinovitch, S.D., 1983)

d. Malignant and Benign Tumors

Strain	Number	Dosage(day)	Method	Age	Dosage(day) mg/m^2
NMR1	75m + 75F	1 ppm	diet	lifespan	5.2×10^{-1}

Animals affected/Total animals

Results: Tumors	56/75m	48/75f
Malignant tumors	38/75m	26/75f
Liver cells, benign	2/75m	0/75f
Pituitary, benign	0/75m	4/75f

Strain	Number	Dosage(day)	Method	Age	Dosage(day) mg/m^2
NMRI	75m + 75f	10ppm	diet	lifespan	5.2

Animals affected/Total animals

Results: Tumors	47/75m	59/75f
Malignant tumors	26/75m	46/75f
Liver cells, benign	3/75m	0/75f
Liver cells, malignant	1/75	0/75f
Pituitary, benign	2/75m	6/75f

Strain	Number	Dosage(day)	Method	Age	Dosage(day) mg/m^2
NMRI	75m + 75f	100ppm	diet	lifespan	5.2×10^{1}

Animals affected/Total animals

Results:	Tumors	56/75m	55/75f
	Malignant tumors	32/75m	38/75f
	Liver cells, benign	2/75m	0/75f
	Liver cells, malignant	1/75m	0/75f
	Pituitary, benign	0/75m	10/75f
Controls:	Tumors	52/75m	60/75f
	Malignant tumors	27/75m	46/75f
	Liver cells, benign	4/75m	0/75f
	Liver cells, malignant	1/75m	0/75f
	Pituitary, benign	1/75	10/75f

Conclusion: There is an unusually high number of tumors seen in the control mice. It is difficult to draw any conclusion about the effects of amitrole. The author does not address the issue of the large number of tumors in the controls.

(Steinhoff,D., Weber, H., Mohr, U. and Boehmes, K. 1983)

2. Rats

a. Toxicity LD_{50} oral 1,100 mg/kg (Registry of Toxic Effects of Chemical Substances, 1980)

b. Thyroid and Liver Tumors

Strain	Number	Dosage (day)	Method	Age	Dosage (day)
White rats	55	20-25mg/rat	drinking water	10-32 months	
	[illegible]	250 or 500 mg/rat	diet	lifespan	6.9×10^3

	Animals affected/Total animals	
Results: Thyroid Tumors	28/55	drinking water
	26/49	diet
Liver Tumors:	15/44	drinking water
	33/52	diet
Hepatocellular and Hepatocholangiocellular	5/44	drinking water
	17/52	diet
Controls: Liver tumours	0/51	
Thyroid cystic adenomas	2/51	

Conclusion: Amitrole induced thyroid tumors as well as liver tumors in 50% of the animals following oral administration in water or diet.

(Napalkov, N.P., 1969 cited in IARC, 1974)

Strain	Number	Dosage (twice weekly)	Method	Age (days)	Dosage(day) mg/m^2
Not Reported	Not Reported	125/mg/rat	Subcutaneous injection	330	9.9×10^2
			Animals affected/Total animals		
Results: Thyroid Tumours			5/7		
Liver Tumours			5/7		

Controls: Not Reported

Conclusion: Amitrole induced thyroid and liver tumors in 71% of the animals.
(Napalkov, N.P. 1962 cited in IARC, 1974)

Strain	Number	Dosage	Method	Age (days)	Dosage(day) mg/m^2
		(3-monthly)			
Not reported	Not Reported	500 mg	pellet implantation, subcutaneous	90	
		then (twice weekly) 125 mg/rat	subcutaneous injection	180	1.8×10^3
			Animals affected/Total animals		Age (days)
Results: Polymorphous cell Sarcoma			1/14		630+
Spindle-cell Sarcomas			6/14		

Controls: Tumors 0/14

Conclusion: Pellets and injections of amitrole induced sarcomas at the treatment site in 50% of the animals. No tumors were observed in the same rats implanted with control pellets or injected only with the sunflower oil medium. It is important to note that amitrole has induced sarcomas in addition to thyroid and liver tumors found in other studies.
(Napalkov, N.P., 1969 cited in IARC, 1974)

Strain	Number	Dosage (day)	Method	Age	Dosage(day) mg/m^2
White Rats	12m	300mg/rat concurrently 2.5/ugT_4/100g b.w.	diet	Not Reported	8.3×10^3

		Animals affected/Total animals
Results:	Thyroid Adenoma	1/12m
	Liver Tumors	9/12m
Amitrole Alone:		
	Thyroid Tumors	7/22 m
	Liver Tumors	12/23m
Controls:	Thyroid Cystic Adenoma	2/51
	Liver Tumors	0/51

Conclusions: Although the thyroid adenomas appear to decrease in number if amitrole and T_4 are concurrently fed, this has no effect on the number of liver tumors found. Therefore, T_4 does not reverse liver tumor formation.

(Napalkov, 1969 cited in IARC, 1974)

c. Thyroid Adenoma and Carcinoma

Strain	Number	Dosage	Method	Age (days)	Dosage(day) mg/m^2
Not reported	10	10ppm	diet	730	4.9
Results:	Thyroid Adenoma		Animals affected/Total animals 1/10		

Strain	Number	Dosage	Method	Age(days)	Dosage (day) mg/m^2
Not Reported	15	50ppm	diet	730	2.5×10^1
Results:	Thyroid Adenoma		Animals affected/Total animals 1/15		
	Thyroid Carcinoma		1/15		

Strain	Number	Dosage	Method	Age(days)	Dosage(day) mg/m^2
Not reported	26	100ppm	diet	730	4.9×10^1
Results:	Thyroid Adenomas		Animals affected/Total animals 13/26		
	Thyroid Carcinomas		4/26		

Conclusion: Amitrole, 100ppm in the diet induced thyroid adenomas in 65% of the animals. Too few animals were considered at risk for the 10ppm and 50ppm amitrole diet to draw any conclusions.

Strain	Number	Dosage	Method	Age(days)
Not Reported	Not Reported	500 pm	diet	119
		normal		14

Results: Thyroid glands were normal, number of animals not reported

Conclusion: There is not sufficient data given to draw any conclusions. However, this study appears to be the Hazelton Laboratory study, details of which are given in section 2e of this table.

(Jukes, T.H. and Shaffer C.B. 1960)

d. Malignant and Benign Tumors

Strain	Number	Dosage(day)	Method	Age	Dosage(day) mg/m2
Wistar	75m + 75f	1ppm	diet	lifespan	4.9×10^{-1}

		Animals affected/total animals	
Results:	Tumors	41/75m	67/75f
	Malignant,tumors	20/75m	34/75f
	Pituitary, benign	9/25	20/75f
	Pituitary, malignant	1/75m	2/75f
	Thyroid,benign	9/75m	12/75f
	Thyroid,malignant	0/75f	1/75f

Strain	Number	Dosage (day)	Method	Age	Dosage(day)
Wistar	75m +75f	10ppm	diet	lifespan	4.9

		Animals affected/Total animals	
Results:	Tumors	44/75m	60/75f
	Malignant,tumors	23/75m	29/75f
	Liver cells, malignant	1/75	0/75f
	Pituitary,benign	0/75m	15/75f
	Pituitary,malignant	0/75	4/75f
	Thyroid, benign	4/75m	8/75f
	Thyroid, malignant	3/75m	4/75f

Strain	Number	Dosage (day)	Method	Age	Dosage(day)
Wistar	75m + 75f	100 ppm	diet	lifespan	4.9×10^1

		Animals affected/Total animals	
Results:	Tumors	53/75m	71/75f
	Malignant, tumors	23/75m	45/75f
	Pituitary,benign	10/75m	36/75f
	Pituitary,malignant	3/75m	5/75f
	Thyroid, benign	45/75m	44/75f
	Thyroid, malignant	18/75m	28/75f

		Animals affected/Total animals	
Controls:	Tumors	36/75m	59/75f
	Malignant, tumors	19/75m	20/75f
	Pituitary, benign	4/75	14/75f
	Pituitary, malignant	1/75m	1/75f
	Thyroid, benign	5/75m	7/75f
	Thyroid, malignant	3/75m	0/75f

Conclusion: There is an unusually high number of tumors seen in the control rats. Yet at 100ppm there is a distinct increase in the number of pituitary and thyroid tumors and a statistically significant, $p \leq 0.06$ decrease in lifespan.

(Steinhoff, D., Weber, H., Mohr, and Boehmes, K., (1983)

e. Enlarged Thyroids

Strain	Number	Dosage	Method	Age(days)	Dosage(day) mg/m^2
Wistar	35m+35F	500ppm	diet	133	
		normal	diet	+49 = 182	2.4×10^2
			Affected animals/Total animals		Age Animals Sacrificed(days)
Results:	Thyroid enlarged and dark or congested		9/9		91
	Kidney - medulla portion congested		3/9		91
Controls:	Areas right lung consolidated		1/10		91
	Kidneys, very much enlarged		1/10		
			Affected animals/Total animals		Age Animals Sacrificed (days)
Results:	Thyroid enlarged		3/6		147
Controls:			Not reported		
Results:	Thyroid slightly enlarged		3/6		154
Controls:			Not reported		
Results:	Thyroid enlarged		13/48		182
	Thyroid, slightly enlarged		25/48		
	Thyroid, normal		7/48		
	Thyroid, dark in color		1/48		
Controls:	Normal		9/10		
	Lungs, abscessed		1/10		

Conclusion: The authors stated that the hyperplasia and degeneration of the thyroid seen in animals fed 500ppm amitrole was reversed after the animals were placed on control diet for two weeks. Yet their gross pathological finding at this time (147 days) showed 50% of the animals have enlarged thyroids. Further at 182 days, that is 7 weeks after the animals were placed on the control diet 79% had enlarged thyroids. The gross pathology cannot lead one to conclude that the effect of amitrole on the thyroid was reversible. Nor is there any other evidence in the study which leads to this conclusion.

(Hazelton Laboratories, 1959)

3. Hamsters

Strain	Number	Dosage (day)	Method	Age	Dosage(day) mg/m^2
Golden	76m + 76f	1ppm	diet	lifespan	
	76m + 76f	10ppm			
	76m + 76f	100ppm			

Conclusion: There was a trend in life shortening in the golden hamsters fed amitrole. At 100ppm this life shortening was statistically significant, $p \leq 0.002$ for both males and females.

(Steinhoff, D., Weber, H., Mohr, U. and Boehmes, K.1983)

VIII. HUMAN STUDIES

There have been three human studies in occupationally exposed groups, two were retrospective studies of railroad workers and the third an exposure assessment and medical monitoring study.

A. Herbicide Exposure, Mortality and Tumor Incidence in Swedish Railroad Workers.

A retrospective study of a cohort of 348 railroad workers exposed to amitrole for more than 45 days, sometimes in combination with 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (diuron) and to some extent with 3-(p-chlorophenyl)-1, 1-dimethylurea (monuron) was carried out. An open wagon on a track was employed for amitrole spraying. This combination of herbicides confuses the evaluation of the relationship between amitrole exposure and the excess tumor incidence. However within this group in the follow-up period of 5 or more years after initial exposure 7 cancers at a variety of sites were observed, whereas only 1.9, ($p < 0.01$) were expected from the national experience. Within this time frame there were 4 deaths observed from all tumors whereas 1.11,($p < 0.03$) were expected. There were two deaths due to lung cancer included in the amitrole cohort and both men were smokers. The authors were aware of the limitations of this study and stated the results were in agreement with animal data and suggested precautions in using amitrole (Axelson, O. and Sundell, L. 1974).

B. Herbicide Exposure and Tumor Mortality, An Updated Epidemiologic Investigation on Swedish Railroad Workers.

The study described in section A above was followed for six additional years. Within the amitrole in combination possibly with monurone and/or diurone exposure cohort, 5 deaths due to tumors at various sites were observed whereas 3.34 were expected. There were 7 cancers at a variety of sites and the average latency time from exposure to the year of diagnosis was 11.4 years. Therefore there were an excess of deaths caused by a variety of tumors. Due to the possible exposure to several herbicides it is not possible to evaluate the observed excess tumor incidence as clearly caused by amitrole exposure (Axelson, O. et al. 1980).

C. Assessment of Exposure and Medical Monitoring

At the request of Union Carbide Agricultural Products Company, Inc. Dynamac Corporation studied 5 applicators with an exposure time of 2.36 hr/day over a period of 10 work days. The crew members rotated jobs: driver helper-1, sprayer-1, helper-2,

and sprayer-2. The team was trained by Mr. Roy Johnson of Union Carbide. Amitrole was applied by men on foot, using hand-held sprayguns. Dermal exposure was estimated by placing multi-layered pads at locations represencing different body areas: face, neck, chest, abdomen, arms, shoulders and legs. The amitrole was extracted from the pads and quantitated by a colorimetric method. The exposure average was 340 mg/man/day for the 10 days, this can be expressed as 4.86 mg/kg b.w. The efficacy of protective clothing was determined from the total amount of residue in the various layers of the pads. The pads consisted of an external layer of medium-weight cloth, middle layer of light-weight cloth and an inner layer of α- cellulose. The external layer was used to determine the efficacy of protective clothing and the middle layer to estimate penetration through protective clothing to work clothing. The inner layer was used to estimate penetration through 2 layers of clothing. Total residues in the 3 layers were taken to represent exposure of unprotected skin. The risk assessment for protective clothing was based upon exposure to applicators wearing a work shirt over a light shirt and coveralls over light trousers, in this situation men were exposed to 134 mg/day. This amitrole residue level was the sum of: "All head pad layers, inner layers of upper torso pads, middle and inner layers of forearms pads and inner layers of leg and ankle pads"(Union Carbide, 1984). The risk assessment based upon applicator exposure with protective work clothing and 30% dermal absorption based on the rabbit dermal absorption study (Shah,P.V. and Gutherie, F.E. 1977) was very high, 0.028.

Thyroid function, as measured by circulating thyroid and pituitary hormones appeared to be a suitable criterion for the short-term toxicological effect of amitrole. This study showed a change in the level of TSH in the expected direction, an increase of almost 30%. However, the number of subjects, the length of exposure and the length of follow-up are inadequate to demonstrate statistical significance. Likewise the statistical power of this study is far too low to rule out an effect on thyroid function. Thus, the two epidemiological studies were inconclusive. The exposure study showed a high risk to applicators and could not rule out an effect on thyroid function.

IX. RISK ASSESSMENT

We have reviewed a large body of experimental evidence on amitrole including evidence on its performance in short-term carcinogen assays, and a number of bioassays in order to answer the following questions: 1) Is amitrole carcinogenic?; 2) Is amitrole likely to be a carcinogen in humans?; 3) What are the chances of developing tumors among persons exposed to typical doses in controlling weeds?

In doing so, it was necessary to consider a number of scientific questions involving general principles of carcinogenesis, and the mechanisms of neoplastic transformation. A number of arguments in this area have been raised by proponents of the use of amitrole. In Part A, we address the general questions, and specific arguments. In Part B, we make an estimate of the carcinogenic effect of exposure to amitrole at levels typical of those to which humans are exposed.

A GENERAL SCIENTIFIC PRINCIPLES AND MECHANISMS OF CARCINOGENICITY:

1. Carcinogenicity of Amitrole.

A wide-range of experiments show unequivocally that amitrole is a carcinogen. Amitrole caused benign and malignant tumors in a range of laboratory animals including mice, and rats. The amitrole was administered mainly in the diet, but also in water, and in some cases, by subcutaneous injection and pellet implantation. The most common tumor types to appear were liver and thyroid, but some were induced at a range of additional sites. Details are given in sections VII and VII, and in Table I.

Amitrole was tested in a number of short-term assays for presumptive carcinogenicity, with positive results in the majority of tests. It was mutagenic and produced chromosomal aberrations in fungi. Additionally, it transformed cell cultures taken from Syrian golden hamsters; it caused increased binding of DNA to intact bacterial cells in the presence of rat liver extract; it produced positive results in both in vitro and combined in vivo and in vitro cell transformation systems. Amitrole tested negatively in the salmonella assay for point mutations. Details are presented in section VI.

2. Type of Carcinogen and Mechanism of Action.

Proponents of the use of amitrole have contended that its carcinogenic activity is due entirely to its interference with the synthesis of T_3 and T_4. According to this view, the cancers result from the consequent overstimulation of the thyroid and pituitary glands, i.e., from an attempt by the body to compensate for the low levels of the thyroid hormones. High rates of cell proliferation are known to be factors in the induction of some cancers. The evidence concerning the mechanism of action for amitrole is discussed in section III.

For our purposes, the crucial point is that proponents of amitrole use contend that this indirect method of action is the only way in which amitrole causes cancer, and that cancer induction by this method has a threshold. That

is, at sufficiently low doses, there will be no overstimulation of the thyroid and pituitary, and therefore, no risk of cancer induction. Further, it is contended that this threshold is in the neighborhood of 50 ppm feeding (or 24 mg/M^2 daily dose) for rats, as this is the dose at which no excess tumors are detected in some bioassays.

It is the overwhelming consensus of scientists that thresholds cannot, in general, be determined for carcinogens, and even small exposures carry excess cancer risks with them (IRLG, 1979). This conclusion is based on several types of observations. Most important of these is that dose-response curves for carcinogens are "stretched out" over many orders of magnitude, i.e., the dose at which most subjects develop cancer may be a factor of 10^5 greater than the dose at which cancer first appears. In some instances, subjects will not develop cancer, regardless of the dose. This is in sharp contrast to the situation for many other toxic effects where all subjects are affected, and usually at a dose 2 to 10 times that of the dose at which the first subjects are affected. There is overwhelming agreement on the no threshold principle for complete or initiating carcinogens. For many carcinogens, scientists have contended that certain substances, usually "indirect acting carcinogens," "late stage" carcinogens or "non-genotoxic carcinogens," work in a fundamentally different mannner, and therefore, obey the threshold principle; i.e., a no effect level can be determined for these substances.

Concerning the regulation of amitrole, we emphasize the following: first, many scientists do not think it is now possible to distinguish between carcinogens which do or do not obey the threshold principle, and some think that there is no such underlying distinction (Weinstein, I. B., 1983). In many cases, substances act as early stage carcinogens in one system, but as late stage carcinogens in others (Day, W., and Brown, C., 1980). Second, in causing tumors at the liver and organs other than the thyroid and pituitary, amitrole in all respects acted as a classical, complete, direct acting carcinogen. It caused mutations,

and chromosome abnormalities in short-term tests. This indicates that amitrole is probably genotoxic, a characteristic generally ascribed to direct acting carcinogens. Moreover, it caused transformation in cell culture, a system in which it is not likely that thyroid hormones are present, and in which the proposed indirect mechanism probably would not be operative. All plausible indirect mechanisms of carcinogenesis involve hyperstimulation of cell proliferation, a process which probably would not be involved in any of the in vitro assays.

More crucial in this case, is the overwhelming evidence that amitrole causes tumors by direct action at the liver and other sites. It is therefore, not necessary to look in detail at the indirect mechanism. Liver tumors were produced in almost every bioassay, generally with a frequency similar to that of tyroid tumors. In one bioassay, about half the mice had both liver and thyroid tumors (Inness, J. et al., 1969). Since the liver is not an endocrine organ, the indirect mechanism cannot operate. Moreover, amitrole does not overly stimulate cell proliferation in the liver. The only plausible indirect methods of carcinogenesis are hormonal carcinogenesis or some other method of overstimulating cell proliferation.

Most importantly, thresholds cannot be determined from typical bioassays. Proponents of the use of amitrole have claimed that negative results in two bioassays indicate that amitrole has a threshold at a dose level somewhat below 100 ppm (approximately 5mg/M^2 per day). But, neither bioassay supports this conclusion. In a typical bioassay (with 100 subjects and 100 controls) and 5% background tumors, cancer would have to be induced in about 15% of the previously tumor free animals in order to have a reasonable chance of detection. In the study reported by Jukes (Jukes, B., Shaffer, C., 1960), only 30 experimental animals lived long enough to be at risk. Projecting from the Vesselinovitch Study (Vesselinovitch, S., 1983), we would expect only 1 additional cancer in this group. Based on these principles, the number of animals at risk is far too

small to be detected.

In the other set of bioassays cited for this purpose, the three performed by Steinhoff (Steinhoff, D., et al. 1983), many factors make it impossible to use them for determining a threshold. First, the proportion of controls with tumors was in the vicinity of 40%; on purely statistical grounds the high number of background tumors makes cancer detection very difficult. In a typical bioassay, the proportion of animals with tumors would have to be twice as big to be detected when the Steinhoff procedure is followed compared to the typical case with 5% or fewer background tumors. Additionally, the incremental effect of a given dose tends to be much lower at higher doses. This is a special case of the flattening effect discussed below. Also in the bioassay with the rats, there is evidence of a trend of increasing tumors with increasing dose starting at 1 ppm. Finally, in another bioassay, tumors were produced with feeding at 60 ppm (Food and Drug Research Laboratories, Inc., 1981).

3. Applicability of Experimental Results.

Proponents of amitrole have questioned the applicability of the bioassay results on a number of grounds - generally involving the liver tumors. We think that all these grounds are invalid. One claim is that the liver of mice and/or rats is a specially susceptible organ. This claim has been overwhelmingly contradicted by a massive body of evidence. Substances that cause liver tumors in mice and rats generally cause tumors in all species tested (e.g., hamsters, guinea pigs, cats), and cause tumors at many sites other than liver (Tomatis, et al. 1973).

Another objection is that liver tumors appeared at very high doses, i.e., near the maximum tolerated dose (MTD). In fact, it is more difficult to produce tumors at such high doses than at lower doses because of competing causes of death. In part, in many experiments performed at such doses, no tumors are produced, even in cases where tumors appear at lower doses. There is no evidence

f r a sharp jump in the response at high doses as is claimed by some. inally, excess tumors were found at doses, a factor of 10 lower in the bio-assay by Vesselinovitch (Vesselinovitch, 1983).

A third objection is that, some agents cause only benign tumors at the liver, and should be classified as tumorigens rather than as carcinogens. This claim is contradicted by Foulds' general laws of cancer progression, and by a wide body of evidenc showing that carcinogen induced, benign tumors frequently develop into full blown malignant tumors, often without addition of any further carcinogens (Foulds, L., 1964). All malignant tumors have passed through a benign stage. Many benign tumors progress to malignancy even without the administration of further carcinogens. Further, if a carcinogen contributes only to the early development of a cancer, it could still be a dangerous agent, adding much to the cancer burden (Whitemore, A., 1977; Day, W., and Brown, C., 1980; Farber, E., 1984).

A fourth possible objection is that the sarcomas could have been induced by a plastic pellet alone, without the inclusion of amitrole. But, control animals with only the plastic pellets, developed no tumors.

B. RISK ASSESSMENT AND JUSTIFICATION:

In general, the bioassays on amitrole present insufficient evidence for use in a quantitative risk assessment. The most important disqualifying factor is that the doses administered were so high as to be on the flat part of the dose-response curve, i.e., where dose increments produce no or much less than proportional increments in the portion of animals with tumors.

Put in slightly different terms - it is typical for carcinogenesis dose-response curves to go through a linear and a flat portion; on the flat portion a major increment in dose causes little change in the portion of subjects developing cancer. For example, in a typical vinyl chloride bioassay, 17% of the animals might develop tumors at .1 ppm exposure, 28% at -1 ppm, and 55% at 100 ppm and 1,000 ppm (Maltoni, 1975). The possible reasons for the flattening include competition between cell killing and transformation by the carcinogen, general

life shortening by the carcinogen, and coupled competing risks from carcinogens induced causes of death other than cancer (Swartz, J., Riddiough, C., and Epstein, S., 1982).

Risk assessments can be performed with a degree of confidence only if many data points are available, or if there is some additional evidence of being on the linear portion of the curve. It is not possible to make a risk assessment using any other technique without two or more data points.

It has been demonstrated that risk assessments made by linear extrapolations (i.e., using the one hit or one stage model) from high dose often underestimate the cancer risk at lower doses (Swartz, J., Riddiough, C., and Epstein, S., 1982). In a substantial number of cases, this underestimate is by a factor of 100 or more.

In the bulk of the amitrole bioassays, the dose was so high that the exposed animals had a shortened lifespan, and coupled competing risks, e.g., in the Inness experiment many of the mice that developed liver tumors also developed thyroid tumors. These experiments were carried out at doses on the flat portion of the curve, and cannot be used for risk assessment.

A weakness of one bioassay (Jukes, T., and Shaffer, C., 1960), was the small number of animals at risk. In this case, the number was too small to use for the detection of excess cancer. Even more animals are required to produce an accurate risk assessment because it is then necessary to discern trends in the proportion of animals with tumors. Only the Vesselinovitch bioassay is likely to be useful for making a risk assessment.

Below we briefly detail the weaknesses of these experiments.

A. Inness et al., 1969 - Dose used was the MTD; many animals developed both thyroid and liver tumors; over 50% of the animals developed tumors. The one dose used was on the flat part of the curve.

B. Jukes, T., and Shaffer, C., 1960 - At the highest dose, about 75% of the animals developed tumors. This means this dose was on the flat portion of the curve. At lower doses, only a total of 30 animals were at risk.

C. Napalkov, N., 1969 - About 50% of the animals at risk developed tumors; dose was close to MTD. Dose was on flat part of the curve.

D. Steinhoff, D., et al., 1983 - There were background tumors in about 50% of the controls, there was major lifeshortening for hamsters.

Only in the case of the Vesselinovitch study is there any reasonable chance of making a quantitative risk assessment. The dose administered to the mice, 500 ppm (2.6×10^2 mg/M^2 per day), is an order of magnitude below the dose administered in other bioassays where liver tumors were observed. Because of the unresolved questions concerning the mechanism of thyroid and pituitary tumor induction, we did not attempt to use data on tumors in these organs for risk assessment. Liver tumors were observed in 36% of adult males and 18% of adult females Approximately 50 of each species survived long enough to be counted. Tumor incidence in the controls was negligible. Hepatocellular carcinomas and hepatocellular adenomas were combined for purposes of the risk assessment. The majority of the tumors were carcinomas.

For all our calculations, we calculated dose on the basis of dose per surface area per day. Species to species comparisons are all made using this presumed equivalent unit. Many studies have shown that dose expressed as weight of toxic substance administered per surface area per day gives results which are most equivalent for different species for a range of toxic effects, including cancer (Rall, D., 1969). The only better extrapolation rule requires data for two or more species under similar experimental conditions. These are data which are unavailable for this study. Some investigators have also suggested that dose measured on a per lifetime basis in cancer bioassays is a better measure than daily dose for extrapolation between species. This is an unresolved issue. It is important to note that the use of lifetime dose would produce higher risk estimates for humans. We do present results in Table II, using a total of four extrapolation rules, combinations of dose per bodyweight, dose per surface area,

with dose per day, and dose per lifetime. We reiterate that given present knowledge, the dose per surface area per day gives the best results.

Because of the relatively low doses and proportion of animals with tumors, (compared to experiments with dose at MTD), we consider it possible to try to perform a risk assessment from the Vesselinovitch data. In particular, we think it feasible that the 500 ppm dose used by Vesselinovitch could be on the linear portion of the curve. All calculations are based on making a downward, linear extrapolation through the origin, i.e., using the linear or one hit model. The justification for use of this model is that the relatively low doses and proportion of responders suggest that this data point could be on the linear portion of the curve. We used the one hit model, which is a special case of the multistage or Weibull model because:

The multistage model has the best biological base and the best experimental verification of all the models used for risk assessment and downward extrapolation; there are insufficient data points to use another model or another form of the multistage model - the one hit model requires only one data point. The calculation is straightforward. The fraction of subjects with tumors is directly proportional to the dose administered, with the exception of doses where the response is very high. At this point, the probability of developing a tumor is $1-e^{-aD}$ where D is the dose and a is a constant.

The exposure levels were taken from field studies by Dynamac Corporation for a simulated applicator. General exposure was estimated by measuring the amitrole in pads attached to the clothing. We assumed that all significant exposure is dermal. Calculations were performed for 4 sets of conditions as follows: 1) with or without specially protective clothing; 2) full dermal absorbtion or 30% absorbtion.

The results are presented in Table III. The projected human lifetime risks range from 0.31, the worst case in which regular clothing is worn, and 100% dermal

absorption is assumed, to a low of .028, in which protective clothing is worn, and 30% dermal absorption is assumed. Lifetime excess risk calculations are included for three additional doses down to 10^{-2} mg/M^2 per day. These risks are high, but the exposure even to applicators trained by Union Carbide for this study is very high. This extremely high risk exists whether the applicators wore standard or protective clothing. Risks accordingly would be much lower for persons with incidental contact with amitrole.

Our risk estimates are of the same order of magnitude as those made by EPA using the one hit model; i.e., 10^{-1} to 10^{-3} range (E.P.A., 1984). The EPA based its risk assessment on an assay in which only thyroid tumors were observed. As explained above, we thought it inappropriate to base our risk estimates on thyroid tumors alone.

TABLE II

DOSE SUMMARY OF LABORATORY EXPERIMENTS AND ONE HUMAN STUDY FOR AMITROLE *

STUDY	SPECIES	ROUTE OF ENTRY	DAILY DOSE		LIFETIME DOSE	
			mg/M^2	mg/Kg	mg/M^2	mg/Kg
Inness, J. et al, 1969	mice	food	1.04×10^3	3.3×10^2	5.6×10^5	1.75×10^5
Dynamac Corp. 1982 standard dress	human	dermal exposure	2.09×10^2	4.86	5.2×10^5	1.2×10^4
protective clothing			8.9×10	1.9	6.2×10^4	1.4×10^3
Napalkov, 1969	white rats	food	6.94×10^3	1000	5.06×10^6	7.3×10^5
Napalkov, 1967	white rats	subcutaneous	9.92×10^2	1.4×10^2	3.27×10^5	4.7×10^4
Napalkov, 1969	white rats	implant	1.83×10^3	2.6×10^2	6.6×10^5	1.83×10^3
Vesselinovitch, 1983	mice	food	2.6×10^2	8.1×10	1.6×10^5	5.1×10^4
Jukes, T. Shaffer, B., 1960	rats	food	2.4×10	3.5	1.75×10^4	2.6×10^3
Steinhoff, D. et al, 1983	rats	food	4.8	0.7	3.1×10^3	4.9×10^2
	mice	food	0.52	16.2	3.6×10^2	1.13×10^4

*NOTE: Daily dose per surface is preferred unit - others are included for completeness, and because other units might be preferrable in certain situations where several doses were used in an experiment, data are for a representative dose as indicated.

TABLE III

RISK ASSESSMENT TABLE

SITUATION	EQUIVALENT DOSE IN Mg/M^2 PER DAY	SPECIES	LIFETIME EXCESS CANCER RISK	REMARKS
Actual bioassay by Vesselinovitch. Estimates of risk per dose based on this experiment. (Vesselinovitch, 1983)	2.6×10^{2}	Mice	0.30	Feeding experiment Mice fed amitrole for 90 weeks. Proportion of tumors based on average of males and females. Liver tumors only are counted.
Projected lifetime risk based on Dynamac study of amitrole incident on skin (Union Carbide Agricultural Products Co., Inc., 1984).	2.7×10^{2}	Human	0.31	Regular clothing 100% absorption
	8.2×10^{1}	Human	9.4×10^{-2}	Protective Clothing 100% absorption
	8.1×10^{1}	Human	9.3×10^{-2}	Regular clothing 30% absorption
	2.5×10^{1}	Human	2.8×10^{-2}	Protective clothing 30% absorption
Lifetime risk based on varying daily doses.	10	Human	1.1×10^{-2}	
	1	Human	1.1×10^{-3}	
	10^{-1}	Human	1.1×10^{-4}	
	10^{-2}	Human	1.1×10^{-5}	

X. PERSISTENCE IN SOIL AND WATER

The use of amitrole for vegetation management requires critical examination of resulting environmental persistence and contamination.

It is known that amitrole will persist in soil for several weeks (National Toxicology Program, 1983). Microbial breakdown occurs in approximately 2 - 3 weeks in moist, warm soil. The losses due to photo-decomposition and/or volatization are minor. Therefore, the resultant average persistence at the recommended application rate is approximately 2 - 4 weeks (Weed Science Society of America cited in Toxicology Data Bank). Experiments with ^{14}C labelled amitrole confirmed this persistence. A half-life of about 8 days was determined with levels decreasing to nondetectable in approximately 6 weeks (Kearney, P.C. and Kaufman, D., 1975 cited in Toxicology Data Bank). EPA states that amitrole exhibits intermediate soil mobility and is persistent in hydrosoil (EPA, March 1984).

It is of concern that amitrole persists in water for more than 200 days (IARC, 1974; National Toxicology Program, 1983). The EPA states that amitrole is persistent in water. Moreover, as amitrole is soluble in water, it is transported in irrigation water (EPA, March 1984). There is also the additional potential of groundwater contamination.

Therefore, when amitrole is applied it should be remembered that it will remain in the soil for approximately a month and in the water for almost seven times as long.

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